Remarks

Upon entry of the foregoing amendments, claims 68-70, 79-93, 98-100, and 104-124 are pending in the application, with the independent claim being claim 68. Claims 71-78, 94-97, and 101-103 were cancelled, claims 68-70, 79, 98, 112-114, 117, and 119 were amended and claims 121-124 were added.

It is believed that these changes introduce no new matter, and their entry is respectfully requested. Claim 68 was amended to specify that the vector is a vaccinia vector, which is supported throughout the specification. Claims 68, 70, 117, and 119 were also amended to clarify that the host cells need only to "undergo a lytic event" and need not have undergone a complete cell lysis according to the present invention. Rather, according to the invention, an initiation of lysis is all that is necessary for recovery of the host cells and isolating the vector of interest. Support for "undergo" can be found in the specification at page 21, line 14. See, also, Example 3 at page 50, lines 17-26 of the specification, which indicates cells that "underwent a lytic event" via incubation for 30 minutes with ovalbumin peptide-specific CTL were harvested. As the examiner can appreciate, a complete cell lysis may not occur during a 30 minute incubation time with the CTL. Accordingly, for clarity, Applicants have amended the claims to refer to "undergo a lytic event." While the previous terminology, "have undergone a lytic event," also does not necessarily require complete cell lysis, Applicants believe "which undergo a lytic event" to better reflect the intended scope and meaning. The amendment does not narrow the scope of the claims.

Claims 69 and 70 were also amended to recite "said recovered host cells" rather than "those host cells which have undergone a lytic event," in view of the amendment discussed

above. The new phrase has the same meaning as the former phrase, and the amendment does not narrow the scope of the claims.

Claims 79, 98, and 112-114 were amended to change dependency.

Dependent claims 121-124 were added. These claims find support throughout the specification, for example, at page 21, lines 14-15.

Thus, no new matter has been added by way of amendment. Based on the above amendment and the following remarks, Applicant respectfully requests that the Examiner reconsider all outstanding objections and rejections and that they be withdrawn.

Interview

Applicant thanks Examiners DeCloux and Nolan for the courteous and helpful interview extended to Dr. Maurice Zauderer and Applicant's undersigned representative on January 8, 2003.

The Rejection for Lack of Novelty

The rejection of claims 68-70 and 120 under 35 U.S.C. § 102(b) over Wolfel et al., U.S. Patent No. 5,530,096 (the '096 patent) was maintained. (Paper No. 37, p. 2.). Applicant respectfully traverses this rejection. Solely to expedite prosecution, the claims have been amended to recite mammalian host cells and a vaccinia vector. Since all the elements of claims 68-70 and 120 are not present in the '096 patent, the rejection should be withdrawn.

As mentioned above, solely to expedite prosecution, Applicant has amended claim 68 to require a vaccinia vector and mammalian host cells. However, as discussed in detail below, adding these limitations is not necessary as the passage of the '096 patent that the

Examiner indicated at the interview (held January 8, 2003) that she is now relying on (see column 16 of the '096 patent) is, at best, nonenabling and may even be inoperative.

At the interview, Applicant pointed out that, in the '096 patent, recombinant plasmids were first isolated from pools of bacterial clones and were used to transfect eukaryotic (COS-7) cells. The '096 patent, col. 4, lines 40-55. These COS-7 cells were then screened for the ability to stimulate the release of TNF from a specific CTL clone, as measured by adding supernatant to WEHI cells and measuring subsequent WEHI cell lysis. *Id.*, col. 5, lines 5-11. The act recited in claim 68(b) of recovering the actual cells that had reacted with the CTLs was never carried out nor was the act recited in claim 69 of isolating the desired vectors from those cells. Instead, in the '096 patent, once a positively reacting pool was identified, individual plasmids from the earlier-transformed *bacteria* were used to transfect new eukaryotic cells, to repeat the transfection and screening process until a unique clone was identified. *Id.*, lines 21-46. Thus, as discussed at the interview, the method taught in the '096 patent is very different (and much more labor intensive) than the claimed invention which, as indicated above, permits recovering the mammalian host cells themselves that undergo a lytic event and isolating the vectors contained therein.

In previous office actions, Examples 4-6 of the '096 patent (columns 4-5) were relied on by the Examiner to support the rejection. Thus, the above arguments, which were submitted by Applicants at the interview and in the previous reply filed September 4, 2002, were intended to address the Examiner's concerns as stated in the previous office actions.

However, at the interview, the Examiner indicated that column 16 of the '096 patent is now being relied on to support the rejection. In particular, at column 16, the '096 patent states:

> Essentially, the method involves identifying a cell which is the target of a cytolytic T cell of the type discussed supra. Once such a cell is identified, total RNA is converted to a cDNA library, which is then transected into a cell sample capable of presenting an antigen which forms a complex with a relevant HLA molecule. The transfectants are contacted with the CTL discussed supra, and again, targeting by the CTL is observed (lysis and/or TNF production). These transfectants which are lysed are then treated to have the cDNA removed and sequenced. . . .

'096 patent, col. 16, lines 17-26. Applicant respectfully submits that this passage, which is prophetic and not supported by experiment, is, at best, nonenabling and, quite possibly, would be inoperative if, in the next office action, the Examiner intends on taking the position that lysed cells, including recombinant pcDNAI plasmid (pcDNAI is the only vector Applicant can find that is described in the '096 patent), could be recovered separately from intact, unlysed cells by following the very sketchy language disclosed at column 16 of the '096 patent. One problem with such a position would be that, as part of lysis mediated by cytotoxic T cells, the target cells undergo apoptosis, including fragmentation of nuclear DNA. Thus, transfected plasmid DNA (e.g., such as pcDNAI), which must localize to the nucleus for expression, may be destroyed in the nucleus along with host cell chromosomal DNA.

The '096 patent does not acknowledge this pitfall associated with using plasmids, such as pcDNAI, which must localize to the nucleus for expression. Importantly, not only does the '096 patent not disclose this pitfall, but further, no guidance is provided in terms of a solution. Thus, at best, the method prophetically disclosed at column 16 of '096 patent is nonenabling. This pitfall is not a problem in the method of the present invention which utilizes vaccinia vectors packaged in the cell cytoplasm, which the present inventor has shown can be recovered as infectious particles even from cells that undergo a lytic event.

Moreover, even if it is assumed, arguendo, that the pcDNAI recombinant plasmids might possibly survive in host cells that undergo a lytic event, no guidance is provided in column 16 of the '096 patent concerning how the DNA could be isolated. Indeed, at column 16, lines 25-26, the '096 patent merely states that "[t]hese transfectants which are lysed are then treated to have the cDNA removed and sequenced." No details or guidance is provided concerning how the lysed transfectants should be "treated." One of ordinary skill in the art wanting to isolate DNA by, for example, PCR would face at least two problems. First, amplification of plasmid DNA in the presence of high concentrations of complex host cell chromosomal DNA is very inefficient because of random binding of primers to the complex sequences present in total cellular DNA. Second, amplification of plasmid DNA inserts or of a plasmid DNA fragment does not recreate an independent genetic unit capable of replication. If a plasmid such as pcDNAI is used, the isolated amplified DNA must be reintroduced into an intact biological vector. This is in contrast to recovery of vaccinia virus recombinants according to the present invention which are replication competent and can be immediately employed for further cycles of selection or for amplification by replication in fresh host cells.

The pending claims are therefore novel over the cited patent. Accordingly, reconsideration and withdrawal of the rejection are respectfully requested.

The Obviousness Rejection

Claims 68-111, 114-115 and 117-120 were rejected under 35 U.S.C. § 103 (a) as being obvious over Wolfel et al. (the '096 patent) in view of Paoletti et al. (U.S. Patent 5,494,807) (the '807 patent).

As discussed above, the '096 patent does not disclose the subject matter of claim 68, which has been amended to recite mammalian host cells and vaccinia virus. Moreover, the '096 patent is at best nonenabling, and may even be inoperative for the method prophetically disclosed at column 16, as discussed in detail above. The '807 patent fails to cure the defects of the '096 patent. Applicant could not find in the '807 patent any guidance for carrying out the prophetic disclosure at column 16 of the '096 patent. Nor did the '807 patent contain a teaching or suggestion to use vaccinia virus to produce a *library* of nucleic acids, as recited in the claims. The '807 patent merely describes the deletion from vaccinia virus of regions encoding known or potential virulence factors, and the insertion of previously isolated genes into the vaccinia virus deletion constructs.

Moreover, neither the '096 patent nor the '807 patent discloses a library produced in vaccinia virus using the method recited in claim 98. This method ("tri-molecular recombination") involves *in vivo* homologous recombination between two fragments (arms) of the vaccinia virus genome and DNA containing an insert. The insert is flanked on one side by a sequence having homology with one arm and on the other side by another sequence having homology with the other arm. After transferring the three DNA molecules into a cell, they recombine to produce a viable vaccinia virus recombinant containing the insert.

The pending claims are therefore nonobvious over the cited patents. Accordingly, reconsideration and withdrawal of the rejection are respectfully requested.

Conclusion

All of the stated grounds of objection and rejection have been properly traversed, accommodated, or rendered moot. Applicant therefore respectfully requests that the Examiner reconsider all presently outstanding objections and rejections and that they be withdrawn. Applicant believes that a full and complete reply has been made to the outstanding Office Action and, as such, the present application is in condition for allowance. If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided.

Prompt and favorable consideration of this Amendment and Reply is respectfully requested.

Respectfully submitted,

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Version with markings to show changes made

In the Claims:

Please cancel claims 71-78, 94-97, and 101-103.

Please replace pending claims 68-70, 79, 98, 112-114, 117, and 119 with the following claims 68-70, 79, 98, 112-114, 117, and 119:

68. (twice amended) A method for selecting a nucleic acid molecule encoding a target epitope of cytotoxic T-lymphocytes, comprising:

(a) contacting mammalian host cells with cytotoxic T-lymphocytes specific for said target epitope under conditions wherein a host cell expressing said target epitope undergoes a lytic event upon contact with said T-lymphocytes; wherein said host cells comprise a library of heterologous nucleic acid molecules, at least one of said heterologous nucleic acid molecules encoding said target epitope, wherein said library is constructed in a vaccinia virus vector which expresses said target epitope in said host cells, wherein said host cells express a defined MHC molecule, and wherein said cytotoxic T-lymphocytes are restricted for said MHC molecule; and

(b) recovering those host cells which undergo ~~have undergone~~ a lytic event.

69. (once amended) The method of claim 68, further comprising isolating said vector from said recovered ~~those~~ host cells ~~which have undergone a lytic event~~.

70. (twice amended) The method of claim 68, further comprising:

(a) isolating said vector from said recovered ~~those~~ host cells ~~which have undergone a lytic event~~;

(b) transferring said vector to a population of mammalian host cells, wherein said vector expresses said target epitope in said host cells, and wherein said host cells express a defined MHC molecule;

(c) contacting said host cells with cytotoxic T-lymphocytes specific for said target epitope and restricted for said MHC molecule, under conditions wherein a host cell expressing said target epitope will undergo a lytic even upon contact with said T-lymphocytes; and

(d) recovering those host cells which undergo ~~have undergone~~ a lytic event.

79. (twice amended) The method of claim 68 ~~76~~, wherein said vector further comprises a transcriptional control signal in operable association with said heterologous nucleic acid molecules, and wherein said transcriptional control signal functions in a vaccinia virus ~~poxvirus~~.

98. (once amended) The method of claim 68 ~~75~~, wherein said library is constructed by a method comprising:

(a) cleaving a vaccinia ~~an isolated linear DNA~~ virus genome to produce a first viral fragment and a second viral fragment, wherein said first fragment is nonhomologous with said second fragment;

(b) providing a population of transfer plasmids comprising said heterologous nucleic acid molecules flanked by a 5' flanking region and a 3' flanking region, wherein said 5' flanking region is homologous to said first viral fragment and said 3' flanking region is homologous to said second viral fragment; and wherein said transfer plasmids are capable of homologous recombination with said first and second viral fragments such that a viable virus genome is formed;

(c) introducing said transfer plasmids and said first and second viral fragments into a host cell under conditions wherein a transfer plasmid and said viral fragments undergo *in vivo* homologous recombination, thereby producing a viable modified virus genome comprising a heterologous nucleic acid molecule; and

(d) recovering said modified virus genome.

112. (twice amended) The method of claim 98 ~~103~~, wherein said vaccinia virus genome comprises a modified thymidine kinase (tk) gene which comprises a 7.5k promoter, a unique NotI restriction site, and a unique ApaI restriction site.

113. (twice amended) The method of claim 98 ~~103~~, wherein said vaccinia virus genome comprises a modified thymidine kinase (tk) gene which comprises a synthetic early/late (E/L) promoter, a unique NotI restriction site, and a unique ApaI restriction site.

114. (twice amended) The method of claim 98 ~~103~~, wherein the 5' and 3' flanking regions of said transfer plasmids are capable of homologous recombination with a vaccinia virus thymidine kinase gene.

117. (once amended) The method of claim 68, wherein said host cells are a monolayer, and wherein those host cells which undergo ~~have undergone~~ a lytic event are released from said monolayer.

119. (once amended) The method of claim 70, wherein said host cells are a monolayer, and wherein those host cells which undergo ~~have undergone~~ a lytic event are released from said monolayer.

Please add new claims 121-124.